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# Effect of Low-Power Laser Irradiation on the Mechanical Properties of Bone Fracture Healing in Rats

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***Background and Objective:*** **Low-power laser irradiation (LPLI) has been found to have a positive effect on bone fracture healing in animal models, based on morphogenic, biochemical, roentgenographic, and electron microscopic measurements. We investigated the effect of LPLI on bone fracture healing in rats using biomechanical methods.**
***Study Design/Materials and Methods:*** **Two groups of male Wistar rats, divided in a randomized block design in a blinded fashion, each consisting of 25 animals, were subjected to anesthesia and tibial bone fracture with internal fixation. The first group was treated with LPLI (HeNe laser 632.8 nm, 35 mW), applied transcutaneously over 30 minutes to the area of the fracture daily for 14 days. The second group served as a control. After 4 weeks, the tibia was removed and tested at tension up to failure (by a Lloyd LR 50K testing apparatus, U.K.) in 16 rats from group I and 15 from group II. The maximal load at failure, the structural stiffness of the tibia (callus stiffness), and the extension maximal load were measured.**
***Results:*** **The maximal load at failure and the structural stiffness of the tibia were found to be elevated significantly in the irradiated group ($P = .014$ and $P = .0023$, respectively), whereas the extension maximal load was reduced ($P = .015$). In addition, gross non-union was found in four fractures in the control group, compared to none in the irradiated group.**
***Conclusion:*** **These results suggest that LPLI treatment may play a role in enhancing bone healing. Lasers Surg. Med. 22:97–102, 1998.** **© 1998 Wiley-Liss, Inc.**

**Key words: bone healing; low-power laser; biomechanical properties**

## INTRODUCTION

Fracture healing may be modified by several factors such as hormones, vitamins, minerals, local vascularity, weight bearing, protein diet, ultrasound, and electrical stimuli [1–5]. Recently, some attention has been paid to another kind of potential stimulating factor, low-power laser irradiation (LPLI). Some promising results have been reported: Using a transmission electron microscope, Tang and Chai [6] found that laser irradiation on rat bones enhanced the activity of red blood cells, macrophages, fibroblasts, chondrocytes, and osteoclasts within the fracture area. Trelles and Mayayo [7] used LPLI on tibiae in mice and, using an optic microscope, observed an increase in vascularization and faster formation of osseous tissue in the treated animals. Yamada

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[8] showed LPLI to have a positive effect on proliferation, differentiation, and calcification of clonal osteoblastic cells. Barushka et al. [9] found that HeNe laser irradiation on hole injuries in the tibia of the rat affected the population of osteoblasts and osteoclasts by alterations in alkaline phosphate- and tartrate-resistant acid phosphatase activities. They also found that LPLI caused an approximately twofold enhancement in bone repair in the hole injury of the rat tibia, as revealed by histophotometry. Glinkowsky and Rowinsky [10] used low-level diode laser therapy on tibial fractures in mice, and the evaluation of the bone radiographs, by a laser densitometer, demonstrated higher optical density in the irradiated group compared to controls.

All of these studies were conducted by using morphogenic, biochemical, roentgenographic, and electron microscopic measurements. Since biomechanical strength and stiffness of a callus are considered as being ultimate indicators of functional recovery of bone fracture, we decided to investigate the effect of LPLI on bone fracture healing in rats, testing the results by means of mechanical parameters.

## MATERIALS AND METHODS

Fifty 4-month-old male Wister rats (weighing 400 ± 20 g) were subjected to anesthesia (ketamine and xylazine intraperitoneally, 125 mg and 7 mg, respectively). A Kirschner wire (1.0 mm diameter) was threaded into the medullary cavity of the right tibia through the skin and patellar ligament over the knee and was advanced down to the distal end of the tibia (Fig. 1). The tibia was exposed by a 0.5-cm longitudinal median skin incision directly over the bone. Using a dental drill, three holes were made at right angles in the midshaft of the tibia. The tibia was then gently broken by light manual bending while the Kirschner wire was held in place in the medullary canal. The incision was closed with interrupted silk sutures [2]. The fifty rats were divided in a randomized block design in a blinded fashion into two equal groups.

Group I was treated with LPLI while under general anesthesia. Immediately after the operation, laser irradiation (HeNe, CW, 632.8 nm, 35 mW) was applied transcutaneously to the shaved skin at the injured area from a distance of 20 cm for 30 minutes daily for 14 consecutive days. The laser was held during irradiation on a stable stand under the supervision of one of the authors.

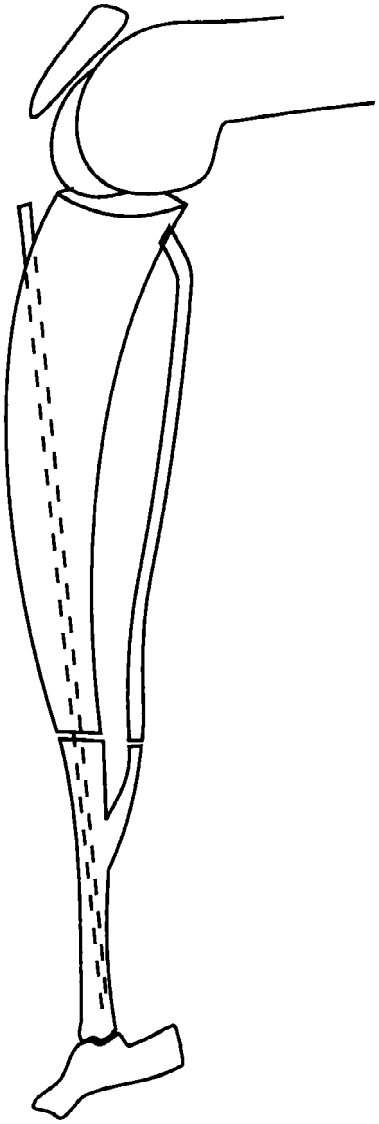


Fig. 1. A schematic example of the rat tibial bone fracture and way of fixation.

The beam was applied to the fractured area for 10 minutes, to the immediate area above the point of fracture for 10 minutes, and to the immediate area below for another 10 minutes. Group II served as controls: These rats underwent the same anesthesia and surgery but no irradiation.

After 4 weeks, the tibiae were removed and immediately immersed in Ringer lactate solution. The animals with infected fractures, malalignment, or gross non-union were excluded from the mechanical study, since they did not exhibit a normal pattern of fracture healing. The decision to examine the callus after a period of 4 weeks is the result of our pilot study: The calluses were checked for failure of distraction at weeks 2, 4,

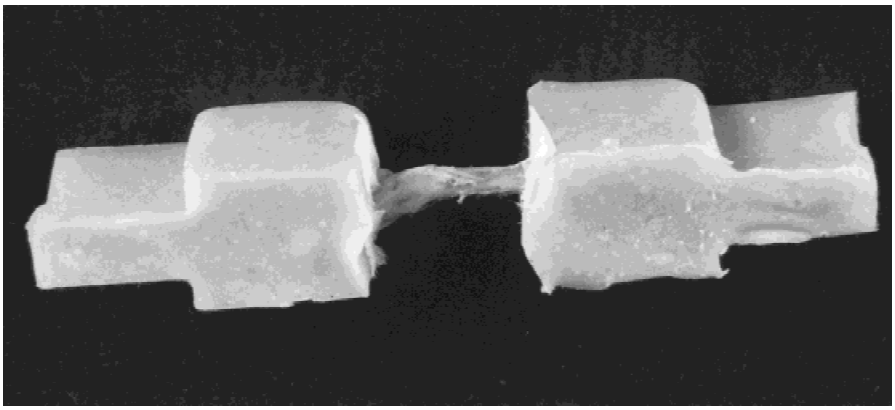


Fig. 2. Methylacrylate holders of bone edges as prepared for mechanical testing.

and 6. At week 2, all the calluses were found to be too immature. At week 4, the callus strength was close to that of the fully healed fracture, and at week 6 the fractures were totally united and exhibited a hard tissue type of behavior, similar to Christel et al. [11]. Both ends of the tibia were then embedded in methylacrylate, in a specially designed jig, to allow a firm grip for the mechanical testing (Fig. 2). The callus area was measured with a caliper followed by an arithmetical calculation, using the equation $S = \pi ab$, assuming that the callus formed an ellipse. The bones were tested at tension up to failure by a Lloyd LR 50K testing apparatus at a cross head speed of 5 mm/min, according to the method used by Christel et al. [3,11] (Fig. 3).

Five parameters describing the status of the callus were studied and obtained by the apparatus numerically and graphically (Fig. 4):

1. The maximum load at failure (in newtons).
2. The stress high load (in newtons/mm$^2$).
3. The extension maximum load (in cm).
4. Structural stiffness of the tibia (callus stiffness) defined as the slope of the straight portion of the force deflection curve before failure (in newtons/mm).
5. The maximum sample area of the callus (mm$^2$).

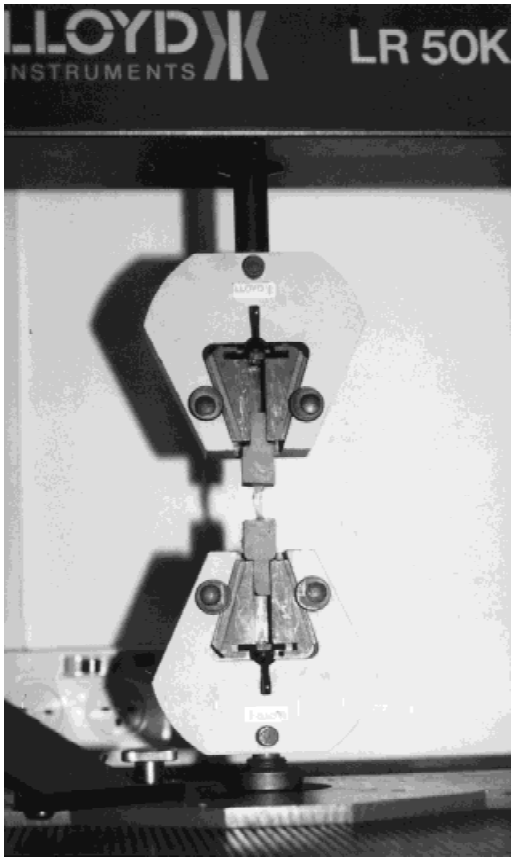


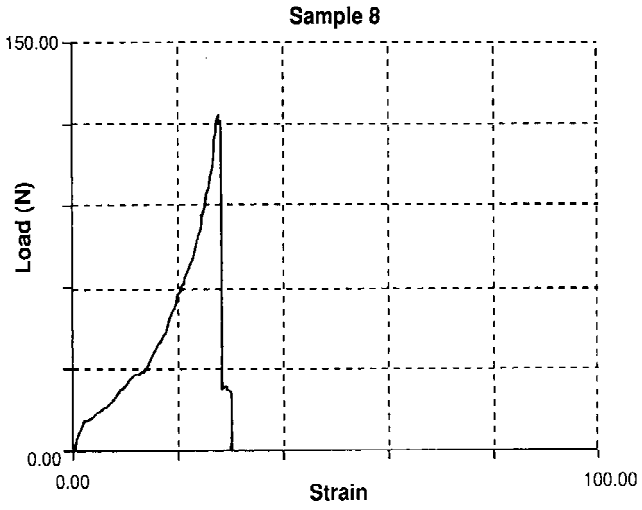

Fig. 3. The rat tibial bone during tension test in the Lloyd LR 50K instrument.

### Statistical Analysis

The results are given as means ± standard deviations. Two-sample t-tests were used in order to compare continuous variables between the two groups. The log transformation was performed due to the large standard deviations of the dependent variables. In order to compare the proportion of nonunion between the two groups a Fisher exact test was done.

## RESULTS

The maximum load at failure was significantly higher in the irradiated group compared to




| Maximum Load | Ext Max Load | Stress Break | Strain Break | Load High Yld | Stress High Yld | Sample Area |
|---|---|---|---|---|---|---|
| (N) 124.0 | (mm) 5.571 | (N/mm$^2$) 1.090 | (%) 29.81 | (N) 10.87 | (N/mm$^2$) | (mm$^2$) 19.60 |

Fig. 4. A typical load–strain graph of a callus subjected to tension stress.

the non-irradiated one (74.4 N ± 43.1 vs. 46.5 N ± 20.2, $P = .014$) (Table 1). On the other hand, the maximum callus area was found to be lower in the irradiated group (19.3 mm$^2$ ± 3.8 vs. 22.9 mm$^2$ ± 5.7, $P = .02$) (Table 1). As a result, the stress high yield was found to be significantly higher in the irradiated group compared to the controls (3.78 N/mm$^2$ ± 2.48 vs. 2.18 N/mm$^2$ ± 1.23, $P < .05$) (Table 1).

The structural stiffness of the tibia (callus stiffness) was higher in the LPLI group (26.28 N/mm ± 11.88 compared to 13.97 ± 10.27, $P = .0023$) (Table 1), whereas the extension maximum load was higher in the control group (4.43 mm ± 2.90 vs. 2.64 mm ± 1.15, $P < .05$) (Table 1). The results of log transformation that have been used because of the large standard deviation are given in Table 1.

Four rats of the irradiation group and two of the control group died before the onset of testing. One of each group had an infected wound. Four irradiated rats and three control rats suffered from malunion due to breakage of the K wires. Four of the remaining 19 rats in the control group had gross non-union compared to *none* of the animals in the irradiated group ($P < .05$) (Table 2).

## DISCUSSION

The effect of LPLI on bone fracture healing in rats was investigated by means of mechanical parameters. Our study showed that the maximum load at failure, the stress high load, and the structural stiffness of the tibia (callus stiffness) were all found to be significantly higher in the LPLI group compared to the non-irradiated control group. The extension maximum load was reduced significantly in the irradiated group, while the maximum callus area showed only a tendency toward reduction compared to the control.

The differences between the properties of the calluses of the two groups indicate that the non-irradiated callus tended to be larger in volume and was also weaker in strength. Perhaps these differences resulted because the callus in the non-irradiated group was more fibrocartilaginous and less ossified in nature (while the bone in the irradiated group had already begun to unite and, therefore, callus was resorbed) as had been shown previously by Trelles and Mayayo [7] and Barushka et al. [9].

Another interesting finding was that gross non-union of fracture at the time of examination (4 weeks post-trauma) was found in four of 19 rats (21%) in the control group but in none of the LPLI group. This may be another parameter which shows the possible positive effect of LPLI on rat bone fracture healing.

As discussed in the Introduction, a positive effect of the use of low-power laser irradiation on bone healing using morphogenic, biochemical, roentgenographic, and electron microscopic measurements was found by others [6–10].

In the current study, we used a biomechanical measurement of the effect of LPLI on bone fracture healing, which is essentially the ultimate proof of real alterations in bone repair—the strength of the healed bone.

The overall dose of irradiation on the skin used in this study (35 mW HeNe laser for 30 minutes, 14 times, i.e., 892 J/cm$^2$) was chosen due to the finding that the skin reduces the energy level of the HeNe laser beam to 3–6% of its original intensity [13]. Thus, we could achieve the energy level of 26 J/cm$^2$ to 52 J/cm$^2$ that was needed to induce proliferation of bone cells, as shown by us in a previous work [12].

David et al. [14] recently concluded that HeNe laser irradiation did not affect bone healing in rats. Based upon our experience, we are of the opinion that they used laser irradiation doses which were too low and that this factor explains the lack of effect.

**TABLE 1. Comparison of Fracture Site Characters Between the LPLI and Control Groups 4 Weeks After the Time of Trauma**

| | Group I (n = 15) (irradiation) | Group II (n = 16) (control) | *P* value (t-test) | *P* value (log transformation) |
|---|---|---|---|---|
| Max load (N) | 74.4 ± 43.1 | 46.5 ± 20.2 | .014 | .032 |
| Callus area ($mm^2$) | 19.3 ± 3.8 | 22.9 ± 5.7 | .02 | .06 |
| Stress high yield ($N/mm^2$) | 3.78 ± 2.48 | 2.18 ± 1.23 | .016 | .01 |
| Extension max. load (mm) | 2.64 ± 1.15 | 4.43 ± 2.9 | .015 | .048 |
| Callus stiffness[a] (N/mm) | 26.28 ± 11.88 | 13.97 ± 10.27 | .0023 | .001 |

[a]Callus stiffness, structural stiffness of the tibia; N, Newton.

**TABLE 2. Complication Rate of Tested Rats (LPLI Group vs. Control)**

| | Total | Died | Infected | Malunion | Non-union |
|---|---|---|---|---|---|
| Group I (irradiation) | 25 | 4 | 1 | 4 | — |
| Group II (control) | 25 | 2 | 1 | 3 | 4 |
| *P* value | | N.S. | N.S. | N.S. | <.05 |

LPLI has been found to modulate other various processes in different biological systems. Rochkind et al. [15–17] showed that LPLI enhances nerve recovery and causes an extension of longevity of cultured myoblast populations. Lubart et al. [18] found that LPLI has a proliferative effect on fibroblasts. Mester et al. [19] and later Galletti et al. [20] found that HeNe laser irradiation accelerates the growth of blood vessels in wound regions as well as the process of cutaneous wound healing. The exact biological mechanism by which LPLI affects tissue healing is not clearly known, but it was recently found that at low radiation doses the light energy is absorbed by intracellular chromophores, such as porphyrins and cytochromes, and converted to metabolic energy involving the respiratory chain via production of a transmembrane electrochemical proton gradient [15,21]. This energy activates metabolic processes, such as an increase in calcium release from mitochondria and ATP production, which enhance and moderate cell activity.

The experimental model in the present study was found to be reproducible and easy to perform. The internal fixation allowed the rats to use their legs soon after surgery. The extent of initial trauma and the shape and site of fracture were well controlled, and the type of fixation we used enabled the micromovements needed for good fracture healing [2,22].

The results of the present study indicate that LPLI plays a role in enhancement of bone healing in rats. Further studies are needed to prove that the same effect exists in humans.

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